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Polymorphism of a Null Allele for AK and MDH Isozymes in the Guppy *Poecilia reticulata*, with a Partial Sex-linked Inheritance

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Summary

Adenylate Kinase (AK) was examined by starch gel electrophoresis for strain differences in the guppy, *Poecilia reticulata*. The electrophoretic patterns revealed two gene loci (*Ak-1* and *Ak-2*) encoding the AK enzyme and the existence of a null allele at the *Ak-2* locus.

Different phenotypic frequencies at *Ak-2* were observed among 16 strains and between females and males within each strain. Such phenomenon has been observed in the *Mdh-4* variants with a mode of partial sex-linked inheritance and *Ak-2* variants which also suggests a mode of partial sex-linked inheritance (both X and Y chromosome). A linkage between *Ak-2 and Mdh-4 locus was revealed.*

Introduction

The guppy, *Poecilia reticulata*, because of its short life cycle, is one of the most useful model for the study of genetics. Sexual dimorphism in which the males show striking color patterns can be used for the study of the mode of inheritance of sex-linked or autosomal genes (1, 2). The establishment of several strains and strain differences can be useful information about the following genetic characteristics; electrophoretic markers (3), growth and reproductive related trains (4), low and high temperature tolerance (5, 6), and salinity tolerance (7).

Fujio *et al.* (5) measured responses to low temperature (12°C, 24h) in different strains of the guppy and indicated a mode of sex-linked inheritance (X chromosome) for resistant (*R*) and sensitivity (*r*) to the low temperature treatment.

Fujio and Macaranas (8) found different phenotypic frequencies at *Mdh-4* among strains and between females and males within each strain and demonstrat-

ed a mode of partial sex-linked inheritance (both X and Y chromosomes) for a null allele.

This work presents evidence that the adenylate kinase (AK) variation is due to a null allele which follows a mode of partial sex-linked inheritance with special reference to linkage of *Mdh-4* locus.

Materials and Methods

Sixteen guppy strains, S, S3, S3HL, O, SC, M1, G, A, B, C, D, D1, F, F22, T and T1, were used for studies on the inheritance of electrophoretic markers. The creation of the strains was based on the color pattern of males (G, A, B, C, D, D1, F, T and T1), subdivision of original strains (S, S3, S3HL and F22), originated from wild population (O), and originated from hybrids of different strains (SC and M1). They were maintained in 60 *l* aquaria at reasonable densities of 300-500 individuals per aquarium, depending on the average size of the strain. The fish were kept at a temperature of 23 ± 2°C and fed ground carp pellets twice a day with dried *Daphnia* as a supplement. Willow moss was placed in the aquarium primarily as a hiding place for newborn guppies.

For electrophoretic analysis, the matured guppies were sampled at random from each strain and the samples were kept frozen at -40°C. Each whole body was homogenized with distilled water and centrifuged at 3,500 rpm for 15 min to obtain the supernatant for starch gel electrophoresis.

Electrophresis and staining procedures were based on Fujio (9). Loci were numbered from the most anodal as *Aat-1* and so on, and alleles were designated as *A*, *B*, *C* from the most anodal downwards. In the present study, the *Ak-2* and *Mdh-4* variants were designated as *A* and *a* to denote a null allele.

Results

Fig. 1 shows the expression of AK isozyme in whole body homogenates of the guppy. Two types of phenotype were observed in AK isozymes; one type exhibited a two banded pattern and the other one exhibited a one banded pattern. Two loci, *Ak-1* and *Ak-2*, were assumed to control activity of the AK enzyme. The *Ak-1* locus was monomorphic for all strains, while the *Ak-2* locus exhibited variation, which was evidenced by single and null bands. This absence of activity was observed in the O, SC, M1, G, A, B, D, D1, F, F22, T and T1 strains, but was not observed in the S, S3, S3HL and C strains. Table 1 shows the phenotypic distribution at *Ak-2* in 16 guppy strains where A designates the banded phenotype and a designates the null phenotype. The frequencies of A and a varied among strains as well as between females and males. Similar phenomenon were observed at *Mdh-4* locus as shown in Table 2. The frequencies

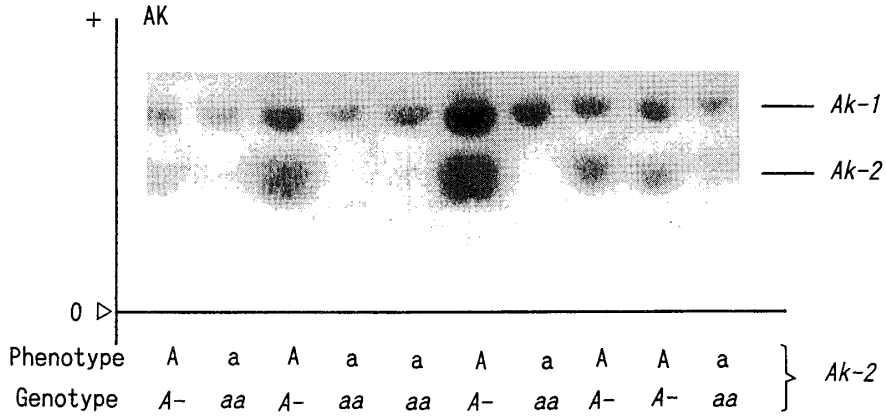

FIG. 1. The expression of AK isozyme on the starch gel electrophoresis.

TABLE 1. *Phenotypes of Ak-2 Variants for 16 Strains of the Guppy*

Strain	Female			Male		
	No. of Fish	Phenotype		No. of Fish	Phenotype	
		A	a		A	a
S	25	25	0	25	25	0
S3	32	32	0	34	34	0
S3HL	60	60	0	60	60	0
O	47	44	3	32	30	2
SC	35	33	2	35	35	0
M1	52	49	3	50	50	0
G	27	23	4	31	30	1
A	56	51	5	64	64	0
B	73	62	11	30	29	1
C	31	31	0	67	67	0
D	70	49	21	15	12	3
D1	36	29	7	44	41	3
F	63	56	7	36	33	3
F22	17	16	1	35	35	0
T	70	63	7	41	36	5
T1	52	34	18	52	39	13

of A and a varied among strains as well as between females and males. Different phenotypic frequencies at *Mdh-4* between females and males demonstrated a mode of a partial sex-linked inheritance (8). Therefore, different phenotypic frequencies at *Ak-2* between females and males suggested a mode of a partial sex-linked inheritance in comparison with no differences between females and males in the gene frequencies of the *Aat-1*, *Pgm-1*, *Pgm-2* and *Sod-2* locus in all

TABLE 2. *Phenotypes of Mdh-4 Variants for 16 Strains of the Guppy*

Strain	Female			Male		
	No. of Fish	Phenotype		No. of Fish	Phenotype	
		A	a		A	a
S	25	24	1	25	25	0
S3	51	35	16	68	55	13
S3HL	60	55	5	60	60	0
O	48	45	3	32	30	2
SC	35	33	2	35	35	0
M1	52	33	19	50	49	1
G	27	21	6	31	30	1
A	56	49	7	64	64	0
B	73	62	11	30	29	1
C	38	26	12	74	69	5
D	70	38	32	15	15	0
D1	36	19	17	44	44	4
F	63	56	7	36	27	9
F22	17	13	4	35	34	1
T	70	67	3	41	41	0
T1	52	29	23	52	34	18

strains (Table 3).

Genotypes of phenotype A were X^AX^A, X^AY^A, X^AX^a, X^AY^a and X^aY^A, while genotypes of phenotype a were X^aX^a and X^aY^a.

The gene frequencies of the *A* and *a* allele on the X and Y chromosomes of 16 guppy strains were calculated based on the frequency of the phenotype a in both females and males. The frequency of the null allele *a* on the X chromosome was calculated by the formula, $qa=\sqrt{a_f/N_f}$, where a_f is the number of a in females and N_f is the total number of females. On the other hand, the frequency of the null allele *a* on the Y chromosome was calculated by the formula, $qa=(a_m/N_m)/\sqrt{a_f/N_f}$, where a_m is the number of a in males and N_m is the total number of males.

As shown in Table 4, the null allele at the *Ak-2* locus was detected in both X and Y chromosomes of the O, G, B, D, D1, F, T and T1 strains and in only X chromosome of the SC, M1, A and F22 strains, but was not detected in both X and Y chromosomes of the S3, S3HL and C strains. The O, F and T strain had the same frequency of null allele in both X and Y chromosomes. The highest frequency of the null allele on the X chromosome was observed in the T1 strain ($qa=0.588$), and the highest frequency on the Y chromosome was also observed in the T1 strain ($qa=0.425$).

TABLE 3. *Gene Frequencies of Isozyme Variants for 16 Guppy Strains*

Strains	Locus	No. of Fish	Female		No. of Fish	Male		Difference between females and males
			q*A*	q*B*		q*A*	q*B*	
S	*Aat-1*	25	0.900±0.042	0.100±0.042	25	0.760±0.060	0.240±0.060	—
	Pgm-1	25	0.300±0.065	0.700±0.065	25	0.280±0.063	0.720±0.063	—
S3	*Aat-1*	51	0.343±0.047	0.657±0.047	68	0.331±0.040	0.669±0.040	—
	Pgm-1	51	0.608±0.048	0.392±0.048	68	0.669±0.040	0.331±0.040	—
S3HL	*Pgm-1*	60	0.217±0.038	0.738±0.038	60	0.225±0.038	0.775±0.038	—
	Sod-2	60	0.208±0.037	0.792±0.037	60	0.192±0.036	0.808±0.036	—
O	*Aat-1*	48	0.708±0.046	0.292±0.046	32	0.625±0.061	0.375±0.061	—
	Pgm-2	48	0.862±0.035	0.138±0.035	32	0.906±0.036	0.094±0.036	—
SC	*Aat-1*	35	0.200±0.048	0.800±0.048	35	0.271±0.053	0.729±0.053	—
	Pgm-1	35	0.571±0.059	0.429±0.059	35	0.457±0.060	0.543±0.060	—
M1	*Aat-1*	52	0.365±0.047	0.635±0.047	50	0.380±0.049	0.620±0.049	—
	Pgm-1	52	0.577±0.048	0.423±0.048	50	0.570±0.050	0.430±0.050	—
G	*Aat-1*	27	0.963±0.026	0.037±0.026	31	0.935±0.031	0.065±0.031	—
	Sod-2	27	0.037±0.026	0.963±0.026	31	0.032±0.022	0.968±0.022	—
A	*Aat-1*	56	0.759±0.040	0.241±0.040	64	0.766±0.037	0.234±0.037	—
B	*Aat-1*	73	0.623±0.040	0.377±0.040	30	0.567±0.064	0.433±0.064	—
	Pgm-2	73	0.747±0.036	0.253±0.036	30	0.763±0.054	0.233±0.054	—
C	*Aat-1*	38	0.658±0.054	0.342±0.054	74	0.662±0.039	0.338±0.039	—
	Pgm-2	38	0.697±0.053	0.308±0.053	74	0.777±0.034	0.223±0.034	—
D	*Aat-1*	70	0.307±0.039	0.693±0.039	15	0.500±0.091	0.500±0.091	—
	Pgm-2	70	0.829±0.032	0.171±0.032	15	0.800±0.073	0.200±0.073	—
D1	*Aat-1*	36	0.375±0.057	0.625±0.057	44	0.307±0.049	0.693±0.049	—
F	*Sod-2*	63	0.190±0.035	0.810±0.035	36	0.194±0.047	0.806±0.047	—
F22	*Sod-2*	17	0.176±0.065	0.824±0.065	35	0.271±0.053	0.729±0.053	—
T	*Pgm-1*	70	0.150±0.030	0.850±0.030	41	0.122±0.036	0.878±0.036	—
	Pgm-2	70	0.821±0.032	0.179±0.032	41	0.805±0.044	0.195±0.044	—
T1	*Pgm-2*	52	0.529±0.049	0.471±0.049	52	0.462±0.047	0.538±0.047	—
	Sod-2	52	0.087±0.028	0.913±0.028	52	0.038±0.019	0.962±0.019	—

TABLE 4. *Gene Frequenhes of Ak-2 Variations for 16 Strains of the Guppy*

Strain	No. of fish	X-chromosome		No. of fish	Y-chromosome		Difference between X and Y chromosome***
		qA	qa*		qA	qa**	
S	25	1.000	0	25	1.000	0	—
S3	51	1.000	0	68	1.000	0	—
S3HL	60	1.000	0	60	1.000	0	—
O	48	0.747±0.044	0.253±0.044	32	0.755±0.077	0.245±0.077	—
SC	35	0.761±0.051	0.239±0.051	35	1.000	0	+
M1	52	0.760±0.042	0.240±0.042	50	1.000	0	+
G	27	0.615±0.066	0.385±0.066	31	0.913±0.051	0.087±0.051	+
A	56	0.701±0.043	0.299±0.043	64	1.000	0	+
B	73	0.612±0.040	0.388±0.040	30	0.911±0.052	0.089±0.052	+
C	38	1.000	0	74	1.000	0	—
D	70	0.452±0.042	0.548±0.042	15	0.635±0.124	0.365±0.124	—
D1	36	0.559±0.059	0.441±0.059	44	0.845±0.055	0.155±0.055	+
F	63	0.667±0.042	0.333±0.042	36	0.750±0.072	0.250±0.072	—
F22	17	0.757±0.074	0.243±0.074	35	1.000	0	+
T	70	0.684±0.039	0.316±0.039	41	0.614±0.076	0.386±0.076	—
T1	52	0.412±0.048	0.588±0.048	52	0.575±0.069	0.425±0.069	+

*: $qa\sqrt{a_f/N_f}$, where a_f presents the number of a in females and N_f presents the total number of females, shown in Table 1.

**: $qa=(a_m/N_m)/\sqrt{a_f/N_f}$, where a_m presents the number of a in males and N_m presents the total number of males, shown in Table 1.

***: Significant difference at a level of $P=0.05$ is indicated by +.

TABLE 5. *Gene Frequencies of Mdh-4 Variations for 16 Strains of the Guppy*

Strain	No. of fish	X-chromosome		No. of fish	Y-chromosome		Difference between X and Y chromosome***
		qA	qa*		qA	qa**	
S	25	0.800±0.057	0.200±0.057	25	1.000	0	+
S3	51	0.440±0.049	0.560±0.049	68	0.659±0.057	0.341±0.057	+
S3HL	60	0.711±0.041	0.289±0.041	60	1.000	0	+
O	48	0.750±0.044	0.250±0.044	32	0.750±0.077	0.250±0.077	−
SC	35	0.761±0.051	0.239±0.051	35	1.000	0	+
M1	52	0.396±0.048	0.604±0.048	50	0.966±0.026	0.034±0.026	+
G	27	0.529±0.068	0.471±0.068	31	1.000	0	+
A	56	0.701±0.043	0.299±0.043	64	1.000	0	+
B	73	0.612±0.040	0.388±0.040	30	0.914±0.051	0.086±0.051	+
C	38	0.438±0.057	0.562±0.057	74	0.800±0.038	0.120±0.038	+
D	70	0.324±0.040	0.676±0.040	15	1.000	0	+
D1	36	0.313±0.055	0.687±0.055	44	0.868±0.051	0.132±0.051	+
F	63	0.667±0.042	0.333±0.042	36	0.249±0.072	0.751±0.072	+
F22	17	0.515±0.086	0.485±0.086	35	0.941±0.040	0.059±0.040	+
T	70	0.793±0.034	0.207±0.034	41	1.000	0	+
T1	52	0.335±0.046	0.665±0.046	52	0.479±0.069	0.521±0.069	−

*: $qa\sqrt{a_f/N_f}$, where a_f presents the number of a in females and N_f presents the total number of females, shown in Table 1.

**: $qa=(a_m/N_m)/\sqrt{a_f/N_f}$, where a_m presents the number of a in males and N_m presents the total number of males, shown in Table 1.

***: Significant difference at a level of $P=0.05$ is indicated by +.

On the other hand, the null allele at the *Mdh-4* locus was observed in all the guppy strains except for the males in the S, S3HL, SC, G, A, D and T strains (Table 5). The highest frequency of the null allele on the X chromosome was observed in the D1 strain ($qa=0.687$), while the lowest was observed in the Strain ($qa=0.200$). The highest frequency of the null allele on the Y chromosome was observed in the F strain ($qa=0.751$).

Discussion

The first discovery of a Y-linked inheritance was demonstrated in the guppy by Schmidt (10) and Winge (11). Two genes *Ir* and *Ma* which control the color pattern were found on the crossing of two strains and were continuously transmitted from father to son, grand son and so on, and nerev to be inherited through the mother. Subsequently, many color genes have been demonstrated by genetic analysis. Some of them were linked with the X and Y chromosomes.

Winge (12) commented that the term "sex-linked" ought to be applied only to the condition of inheritance explainable by the presence of the factor in question in those sex-chromosomes which are normally found in individuals of both sexes (X and Z chromosomes). On the other hand, Yamamoto (13) suggested that all modes of autosomal inheritance are sex-linked. Thus, sex-linked inheritance includes various types of modes and it may depend on which homologus segments are shared by the X and Y chromosomes. When the X and Y chromosome contain at least some homologus segments, genes may be partially sex-linked as demonstrated in the present work.

Furthermore, the possibility of crossing-over between the X and Y chromosomes would suggest that they contain homologus regions, that is, the genes both from X to Y and Y to X cross-over relatively frequently.

Ten years ago, in the S3 strain, the frequency of the null allele at the *Mdh-4* was 0.298 in the X chromosome and 0 in the Y chromosome (8). And in this study, the frequency of the null allele was 0.560 in the X chromosome and 0.341 in the Y chromosome. Similar phenomenon were also observed in the F strain. These results suggest the crossing-over between the X and Y chromosomes. However, such phenomenon were not observed in the SC strain, suggesting no crossing-over between the X and Y chromosomes in this strain. This suggestion was indirectly introduced by the fact that the SC strain originated from the cross between a single male of the cobra type strain and 2 or 3 females of the non-cobra type strain; all SC males were observed to be the phenotype A which mean that the Y chromosome of the SC strain may contain only *A* allele.

To prove whether two kinds of Y chromosomes in the crossing-over between X and Y chromosomes exist or not in the guppy population, it would be necessary to attempt the cross experiments.

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